EFFECTS PRODUCED ON THE PILOSEBACEOUS SYSTEM AND THE ADRENALS OF THE RABBIT BY INUNCTION OF SEX HORMONES*†

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Despite the wealth of literature on the biologic and pharmacologic effects of sex hormones, contributions concerning the changes produced by topical applications are very limited and the results published are by no means concordant. In view of the increasing use of estrogens in ointments, it was deemed of particular interest to investigate not only the local changes brought about by percutaneous application of these hormones, but also to study the systemic effects of such application.

REVIEW OF PREVIOUS ANIMAL STUDIES

A few reports have appeared of experiments on animals indicating absorption of hormones through the skin. Steinach (1) was the first to observe cutaneous hyperemia in rats after local application of estrogens. Almost two decades ago, Zondek (2) showed that estrone was absorbed through the skin of mice and that vaginal cornification could be produced by inunction of seven times the amount which sufficed on injection. Moore, Lamar and Beck (3) studied the absorption of sex hormones through the guinea pig's skin and observed that both testosterone and testosterone propionate, as well as estradiol, were readily absorbed.

A short review of reports of experiments concerned with the influence of sex hormones on the pilosebaceous system follows. Some of the observations are contradictory.

Gardner and De Vita (4) produced leukopenia, anemia, myeloid aplasia and thinning of the hair in dogs by injection of massive doses of estrogens. Williams, Gardner and De Vita (5) applied small amounts (2–6 mg.) of estrone, dissolved in oil, locally in dogs and observed similarly an inhibition of the rate of regrowth of hair. This action is apparently a local effect upon the hair follicles. Wattenwyl (6) implanted estradiol tablets in guinea pigs and produced loss of hair at the site of the implanted hormone and extending for a considerable distance. Removal of the implanted tablet restored normal growth. Arguello (7) observed great diminution in the number of hair follicles in the rat after subcutaneous injection of estrone. On the other hand hyperplasia of the cornified layer of the skin was noticed. Hooker and Pfeiffer (8) found, after subcutaneous injections of estradiol benzoate into rats, a suppression and shagginess of hair growth accompanied by a reduction in size of the sebaceous glands. The animals could be completely protected against these estrogenic effects by the simultaneous injection of androgenic hormone.

Selye (9) in experiments on postpubertal mice (1 male and 3 females), observed an enormous increase of keratin in the hair follicles after 4 months' daily cutaneous application of 250 micrograms of estradiol. Furthermore, he noticed an almost complete disappearance of the sebaceous glands, which he attributed to the pressure from cysts. The latter were considered to be distended hair follicles. Histologic sections of the skin did not show anything else of significance except that the blood vessels of the corium were greatly dilated,

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and that the surface epithelium was more stratified than in untreated controls. It should be emphasized that in spite of the local application of large amounts of estradiol to the bare skin, malignant changes were not induced during the period of observation.

Kun (10), however, reported that estradiol or its benzoate, in an ointment, stimulated growth of hair on infantile and on senile rats in which hair growth is normally poor. Hu and Frazier (11) found that repeated shaving did not alter the rate of hair growth in rabbits, but there was a difference between the sexes. Mature females regrew hair much sooner than did the males of the same litter. In ovariectomized rabbits a retardation of regrowth was noticed, and when males were "estrinified" by the subcutaneous injection of an ether soluble fraction of a butyl alcohol extract from the urine of pregnant women, the initial rate of regrowth of hair was hastened, approaching that of the female rats. However, there was no increase in the subsequent rate of growth. Moreover, the estrogen treated buck showed a retarded rate of growth. Danforth (12) observed that a minute amount of crystalline testosterone propionate placed under the skin of capons would feminize feathers in the immediate vicinity.

Emmens (13) also observed a slower regrowth of hair in rats after parenteral or percutaneous application of estrone, more strongly in males than in females. Androgens did not show any appreciable effect. Female rats, however, exhibited increased hair growth when a saline extract of the female pituitary gland was administered. Male rats remained unaffected. Mulligan (14) also observed loss of hair in dogs after administration of high doses of diethylstilbestrol. In his experiments, the adrenals showed histologic changes in the cortex consisting of shrunken glomerular and fascicular cells and condensation of the nuclear chromatin. Cytoplasmic lipid was lost from the fascicular cells as demonstrated by the scarlet R stain on frozen sections of formaldehized fixed material. In a second dog the epidermis was intact. The epithelium of the hair follicles was thinned, the inner follicular cells showed hyaline cytoplasm and the hair follicles were shrunken. Most sebaceous glands disclosed disappearance of the lipid—laden cells, shriveling of the epithelium and shrinkage. Many sweat glands were dilated and lined with flattened epithelium.

REVIEW OF HUMAN STUDIES

One of the most interesting contributions is that of Hamilton (15) who induced baldness following administration of male hormone substance to sexually immature men "who otherwise would not have become bald". A similar observation was made in castrates and eunuchoid men when they received androgenic medication. Baldness did not progress in either of these instances when the endocrine treatment was discontinued for a year, but "upon further medication thereafter the extension of the alopecia areas was again resumed". This observation has been partially confirmed by Roony and Zakon (16) who demonstrated microscopically, in carefully conducted studies, that methyltestosterone stimulates the sebaceous glands of males and that diethylstilbestrol has a depressive effect on the sebaceous glands of the adult male. The observation of these authors (17) on the influence of androgens upon the sebaceous glands of the pubic region of prepubertal boys is similar.

EXPERIMENTAL

The studies reported here were concerned with the local and systemic effects of percutaneously applied sex hormones. The neutralization of estrogenic effects by androgens, and vice versa, was demonstrated by a simple procedure. Observations were made of the effect of repeated inunction upon the rate of growth of the hair. The effects on the skin and adrenal glands were studied histologically. Attention was also paid to the possible carcinogenic effect of the sex hormones and possible tumor formation.

METHODS

The experiment was conducted on normal and on castrated rabbits. The rabbits used were 3–8 months old and came from the same farm and were kept on a diet composed of Purina, rabbit chow and carrots.

At the start of the experiment, the abdomen of each animal was shaved and two grams of ointment rubbed into the left half of the shaved area. These inunctions were made six times a week. Control rabbits were similarly rubbed with 2 grams of the respective ointment bases. At intervals of three weeks the abdomens of the rabbits were shampooed and the border, surrounding a 24 square inch area, was clipped and the hair discarded. After this, the hair from the 24 square inch area, was clipped, collected and dried to constant weight in an oven at 56°C.

The ointments used were progynon (alpha estradiol), each gram containing 0.15 mg. of estradiol, and oreton (methyl testosterone) each gram containing 2 mg. of methyl testosterone.

After 9 weeks, 4 rabbits which had been receiving progynon inunctions, were changed to oreton and similarly 4 rabbits on oreton were changed to progynon. The experiment lasted 18 to 24 weeks and 6 to 8 weighings of hair were made in most of the animals.

RESULTS

The results are summarized in Tables 1 and 2. These tables indicate an increased weight of the hairs from a clipped area 24 inches square when progynon was inuncted, and a decrease in the weight of the hair from a similar area when oreton was inuncted. Four rabbits (nos. 1, 7, 48, 53) were used as controls and neither hormone ointments or their respective bases were inuncted. The mean clipping weight was used as the index and this figure was obtained by totaling the weights of the number of clippings indicated in each case and then averaging the result. The standard error is also listed on these tables and this refers to the plus or minus deviation from the estimated mean in which there is a 2 to 1 chance that the true mean will fall.

In two rabbits treated with progynon, the hair from the inuncted half of the abdomen was collected and weighed sepearately from that on the untreated area. The weights of hair from the two areas were either approximately equal or the weight of hair was greater from the untreated area. This indicates that the effects of the inunctions were systemic and not purely local. Further evidence of systemic estrogenic stimulation was found in the swelling of the nipples on both sides of all rabbits treated with progynon.

HISTOLOGY OF THE SKIN

In order to investigate changes produced in the skin by the topical application of these hormones, biopsies were performed on the treated area of the following non-castrated males: no. 48 (control), no. 43 (progynon), no. 42 (oreton) and the following castrated males: no.7 (control), no. 46 (progynon) and no. 45 (oreton).

TABLE 1

Weight (in milligrams) of clipped hair from rabbits receiving inunctions of hormone ointments and their bases

GROUP NO.	MEDICATION	RABBIT NO.	SEX	AGE	TOTAL WEIGHT OF RABBIT (grams)	MEAN CLIPPING WEIGHT (milligrams)	STANDARD ERROR (°m)	NUMBER OF CLIPPINGS
I	Control	1	Male castrate	8 mos.	3482	328	78	13
		7	Male castrate	8 mos.	2714	361	62	13
		48	Male	8 mos.	2856	824	108	13
		53	Male	6 mos.	2120	164	53	7
II	C→PB	2	Male castrate	8 mos.	2788	C 807	193	5
						PB 1367	205	8
						M 1087		13
		54	Male	6 mos.	2216	C 426	130	4
						PB 683	240	4
						M 554		8
		55	Male	6 mos.	2480	C 664	234	4
						PB 995	298	4
						M 829		8
III	PB→C→PB	37	Female	4 mos.	1342	PB_1 1280	220	7
				+7 wk.	3782	C 1150	170	4
				(no shaving)		PB_2 1492	142	8
						M 1307		19
		41	Male	3 mos.	2391	PB_1 848	113	9
				+8 wk.	3048	C 486	115	5
						PB_2 738	151	6
						M 671		20
IV	O→P→C	38	Female	4 mos.	1872	O 464	315	3
						P 1685	545	4
				+8 wk.	1988	C 540	74	13
						M 896		20
		42	Male	3 mos.	2413	O 128	45	4
						P 1661	488	5
				+2 wk.	1602	C 272	83	5
						M 687		14
V	P→O→C→OB	39	Female	4 mos.	1468	P 1431	635	3
						O 1096	335	4
				+8 wk.	2292	C 1163	344	4
						OB 1093	156	8
						M 1196		19

TABLE 2

GROUP NO.	MEDICATION	RABBIT NO.	SEX	AGE	TOTAL WEIGHT OF RABBIT (grams)	MEAN CLIPPING WEIGHT (milligrams)	STANDARD ERROR (°m)	NUMBER OF CLIPPINGS
	P→O→C→OB	43	Male	3 mos.	2262	P 2271	668	4
						O 1176	303	5
				+8 wks.	2667	C 259	82	4
						OB 352	124	3
						M 1014		16
VI	C→OB	3	Male cast.	8 mos.	3892	C 588	230	5
						OB 707	108	8
						M 647		13
		5	Male cast.	8 mos.	3228	C 260	74	5
						OB 420	91	8
						M 340		13
		56	Male	6 mos.	2738	C 551	148	4
						OB 776	238	4
						M 663		8
VII	OB→C	44	Male cast.	4 mos.	2810	OB 1205	216	7
				+8½ wks.	2621	C 770	145	6
						M 987		13
VIII	O→P→C→OB	45	Male cast.	4 mos.	2203	O 590	225	3
						P 1768	799	4
						C 613	258	5
						OB 624	212	4
						M 861		16
		51	Female cast.	5 mos.	1712	O 625	282	3
						P 1768	799	3
				+8 wks.	2063	C 534	180	4
						OB 728	113	8
						M 914		18
IX	P→O→C→PB	46	Male cast.	4 mos.	2203	P 1912	79	3
						O 999	262	4
				+7 wks.	2322	C 978	224	5
						PB 1079	312	4
						M 1242		16
		52	Female	5 mos.	1712	P 1261	543	3
						O 806	349	3
				+8 wks.	2308	C 923	239	4
						PB 872	126	8
						M 945		18

Code:

C = control
O = oreton
→ change of treatment
\+ no shaving for period listed
PB = progynon base
P = progynon
M = mean (average of figures listed)

The findings were as follows:

Non-castrated male control: no. 48. No remarkable changes in the epidermis; sebaceous glands large, comparable to those observed in castrates after stimulation with androgenic hormones; no activity in the hair bulb.

Non-castrated male inuncted with progynon: no. 43 (fig. 1). No remarkable epidermal changes; a striking reduction in the size of the sebaceous glands; stimulation of the papillae in the form of increased cell-division; development of subsidiary hair follicles.

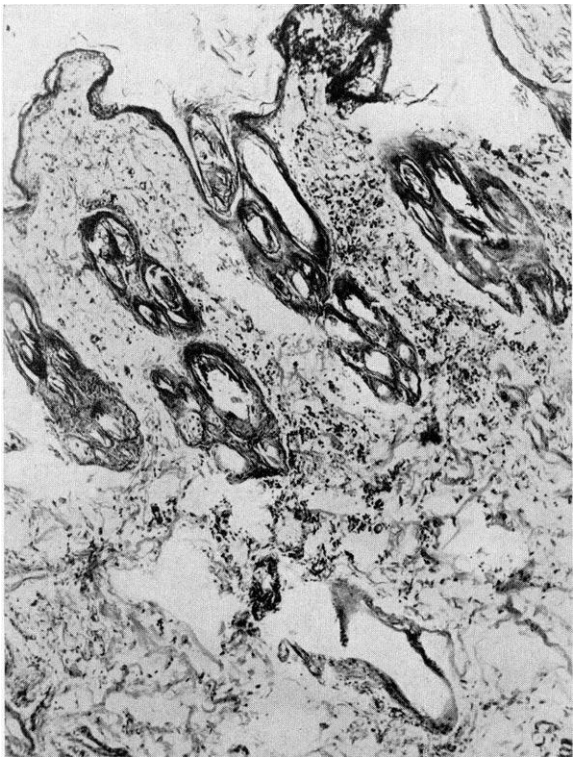

FIG. 1. Section of skin showing reduction in size of the sebaceous glands and development of subsidiary hair follicles.

Non-castrated male inuncted with oreton: no. 42 (fig. 2). Remarkable hyperplasia of the sebaceous glands, without any other noticeable changes in the epidermis.

Castrated male control: no. 7 (fig. 3). Conditions almost similar to non-castrated male treated with progynon, i.e. noticeable reduction of the sebaceous glands and increased activity in hair papillae.

Castrated male inuncted with progynon: no. 46 (fig. 4). Sebaceous glands almost completely disappeared; intensified mitosis in the hair papillae; subsidiary hair papillae developed.

Castrated male inuncted with oreton: no. 45. Changes similar to those in non-catrated male; in addition colloidal degeneration of sebaceous glands with a few pyknotic nuclei.

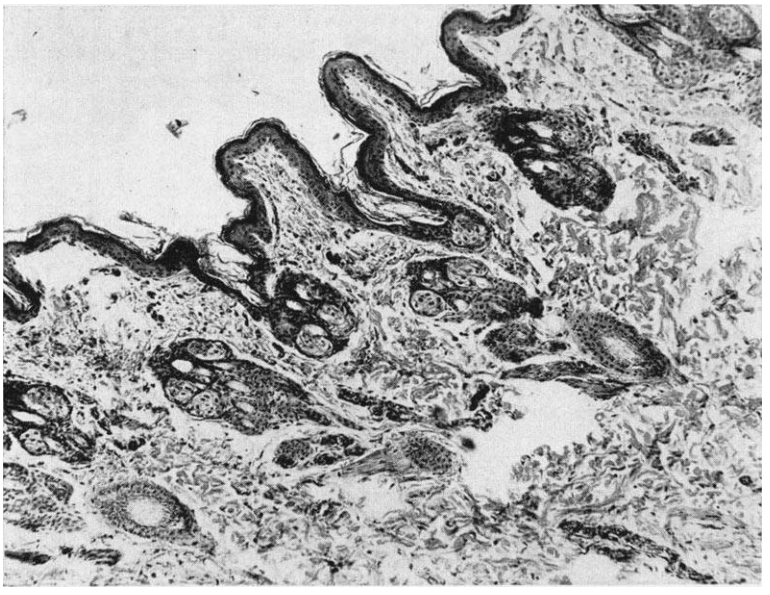

FIG. 2. Section of skin showing hyperplasia of the sebaceous glands and reduced activity of the hair follicles.

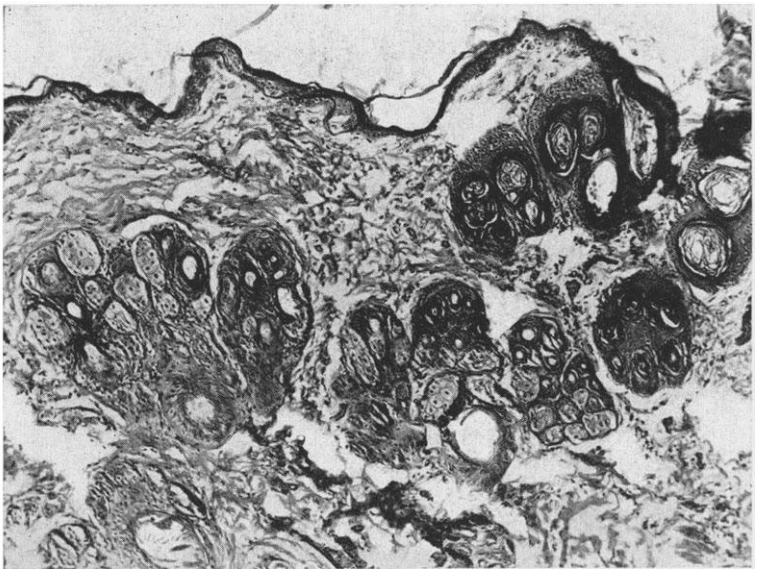

FIG. 3. Section of skin showing reduction of the sebaceous glands.

POST MORTEM STUDY OF THE INTERNAL ORGANS

All animals were finally sacrificed with chloroform. Gross and microscopic examination of the internal organs revealed no changes except in the adrenal glands. The pituitary glands were lost in the laboratory and consequently could

not be examined. Additional experiments are under way and will deal particularly with the pituitary gland. None of the animals showed tumor formation of the inuncted areas or of the mamillae.

HISTOLOGY OF THE ADRENAL GLANDS

In the present study no attempt was made to analyze anatomic or cytologic differences between the adrenals of male, female and castrate animals, though it should be noted that Mazer, Israel and Alpers (18) record hypertrophy of the outer cortical cells and medullary invasion by groups of mid-zonal cortical cells

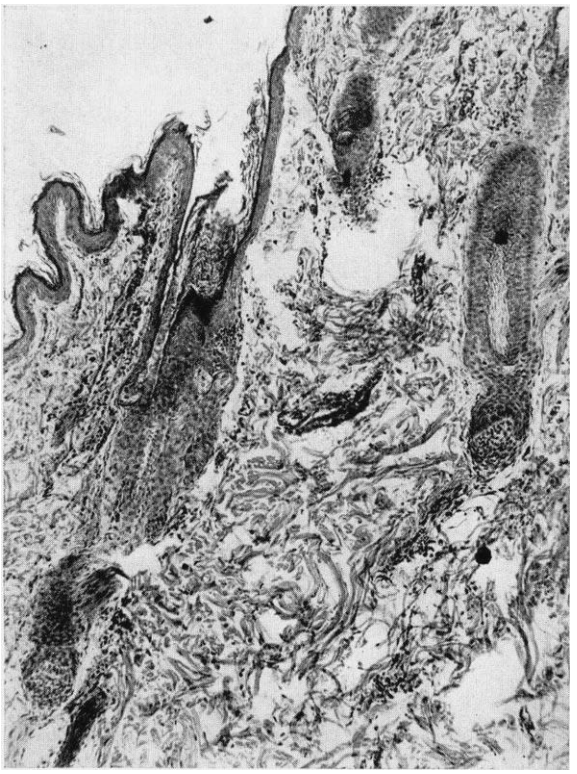

FIG. 4. Section of skin showing almost complete absence of sebaceous glands, increased activity of the hair papillae.

in the adrenals of castrate rabbits. The adrenals of the following castrate and normal males were removed during autopsy: no. 7 (castrated), no. 1 (castrated), no. 2 (castrated), no. 41 (non-castrated), no 42 (non-castrated), no. 43 (non-castrated). Unilateral adrenalectomies were performed on rabbits no. 43 (after 3 weeks daily application of oreton) and no. 46 (4 months after cessation of inunction for 12 weeks with progynon and 9 weeks with oreton). In addition to no. 1 and no. 41, a normal rabbit of the same age, which died during heart puncture, served as a control (no. 50).

The findings were as follows:

Non-castrated male rabbit: no. 48 and no. 50 (fig. 5) (control). A narrow peripheral zone with the cells arranged in circular, glomerular fashion (the glomerular zone) was seen which surrounded a much wider zone of long strands of cells (the fascicular zone). Within this was the reticular zone with cells not arranged in any particular order and finally a zone of variable width of darkly staining cells, arranged in groups (the interlocking zone) was observed within or around the medulla. Frozen sections stained with Sudan III showed the typical staining

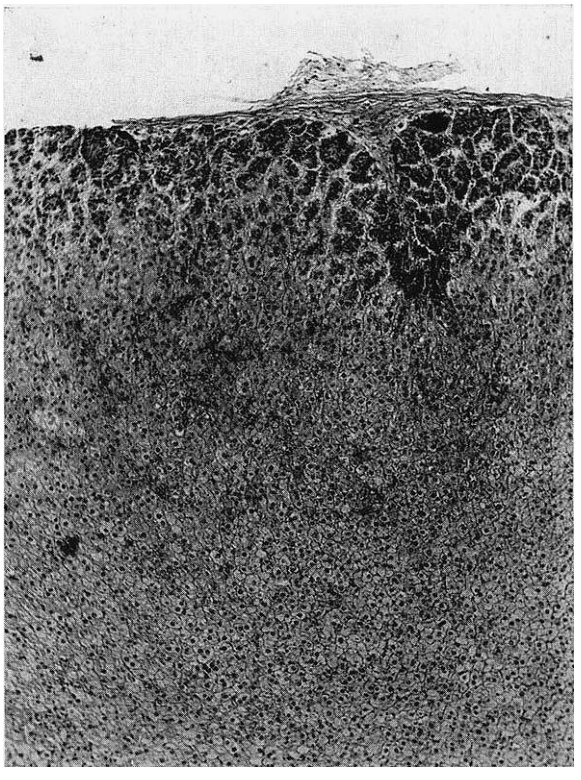

FIG. 5. Section of adrenal gland illustrating normal glomerular and fascicular zones.

reaction for fat which, in the hematoxylin and eosin stained sections, appeared as characteristic lipid vacuolization.

Roaf (19) found a similar lipid deposit in the corpus luteum of rabbits, which he correlated partly with the development of the corpus luteum. In the male adrenal cortex, however, a similar condition exists, which, according to Roaf, "is not understood".

Castrated male rabbit: no. 1, similar to non-castrated male.

Castrate and non-castrate inuncted with progynon: no. 52 (fig. 6) and no. 39. Cells of the glomerular zone arranged in long rows instead of in the normal fashion and stain fainter than the control; increased mitotic activity in the interlocking zone expecially at the border of the medulla. Sudan III stain shows an in-

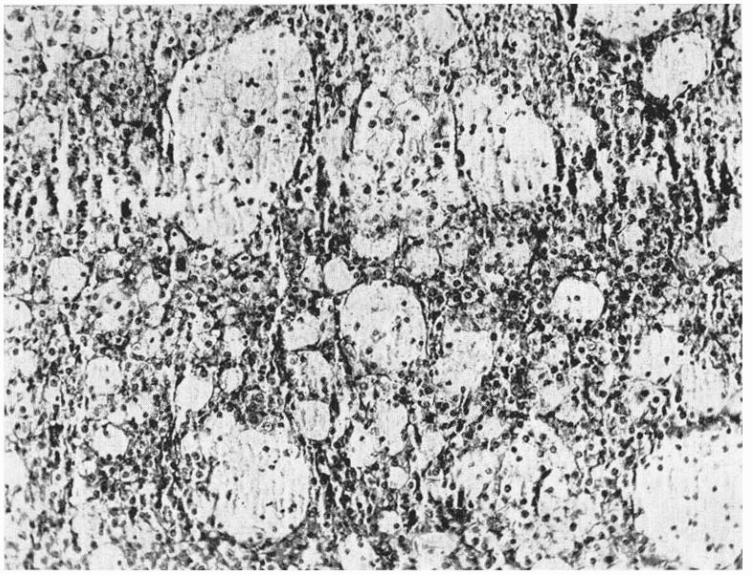

FIG. 6. Section of adrenal gland showing a derangement of the glomerular zone and many large vacuolized cells both in the glomerular and fascicular zones.

FIG. 7. Section of adrenal gland demonstrating a derangement of the glomerular zone with increased lipid deposits in the glomerular and fascicular zones, but to a lesser degree than in the progynon treated animal.

creased fat deposit with many large vacuolized cells permeating almost the encreased fat deposit with many large vacuolized cells permeating almost the entire cortex.

Castrate and non-castrate inuncted with oreton: nos. 45 (fig. 7) and 38. Changes similar to those in progynon treated animals but in lesser degree.

Rabbit no. 46 (fig.8): Four months after cessation of inunctions marked derangement of the glomerular zone with the cells arranged in rows, and increased cellular activity as well as more lipid deposits in the glomerular zone than in the control rabbits.

None of the treated animals showed changes in the medulla.

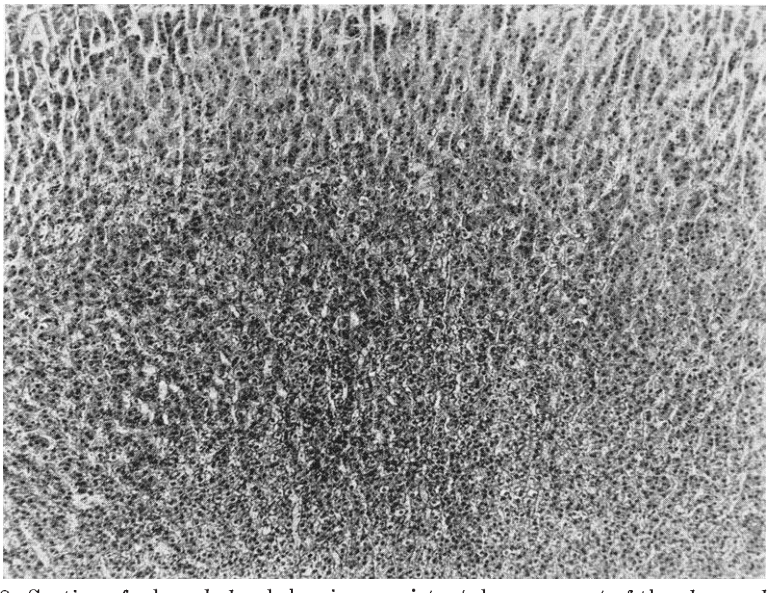

FIG. 8. Section of adrenal gland showing persistent derangement of the glomerular zone.

DISCUSSION

The local application of estrogens and androgens to rabbits' skin by inunction interferes with function of the adrenals as evidenced by histologic studies which showed obvious changes in the cortex. These changes were observed for as long as 4 months after cessation of the hormones but to a lesser degree than in the first month after continuous application. In the rabbits treated with oreton (methyl testosterone) the cortical changes were less pronounced than in those treated with progynon (alpha estradiol).

Mulligan and Becker (20) who experimented on dogs by administering stilbestrol orally also noted changes in the cortex of the adrenal glands and concluded that "the changes seen in the adrenal glands at the height of the action of stilbestrol and the gradual recuperation of the cortex of these structures suggest that the adrenal cortex recovers but not completely, from the injury inflicted on it by stilbestrol." By interpolation, a dynamic picture of the serial recovery of the adrenal cortex may be drawn. Apparently, stilbestrol injures cells of the germinal layers, or zona glomerulosa, so that their function is impaired as they merge with the functional layer, or zone fasciculata. Because of this impaired function,

the cells do not elaborate the amount of lipid normally seen in them. They are injured in such a way that they degenerate earlier into the ballooned cells filled with large globules of fat, ordinarily seen only in the normal zona reticularis or degenerative layer. Because of this earlier degeneration the zona reticularis is greatly widened, relatively devoid of lipid, and concerned only with the removal of degenerated cells from the zona fasciculata, but not with the disposal of their usually abundant remaining lipid. In the process of recovery, the glomerulosal and fascicular zones show gradual widening of the formerly narrowed cortex and regeneration of new functional cells from the germinal layer with an

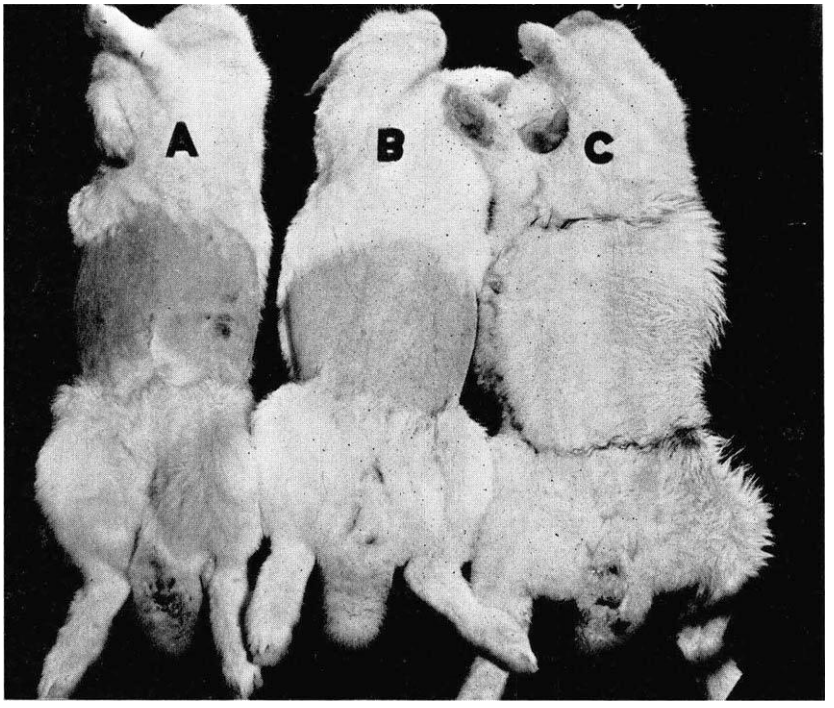

FIG. 9 (a). Normal rabbit (with no treatment) showing poor growth of hair 3 weeks after clipping.
(b) Rabbit treated with oreton showing scanty growth of hair.
(c). Rabbit treated with progynon showing profuse growth of hair.

increasing complement of lipid, so that a condition approaching that of the normal gland is attained.

The changes described above may be indicative only of a temporary and reversible functional alteration of the adrenal cortex but it may indicate that similarly the human adrenal may also be affected after prolonged percutaneous application of sex hormones. Application of results of animal experimentation to humans cannot be made at all times, but since the function of the adrenals in rabbits and humans have a great similarity, some deduction is justified. It should be borne in mind that caution should be exercised in the prolonged clini-

cal use of sex hormone preparations. This statement is of particular importance in view of the histopathologic changes found in castrated and non-castrated rabbits which show changes in the adrenals, although not as severe as those described by Mulligan and Becker, but sufficient to indicate a functional disorganization. It is assumed that such changes may be of only temporary nature. This is confirmed in the lesser changes observed in the castrated male rabbit even 4 months afrer cessation of the inunction.

The hormonal relationship controlling the growth of scalp hair has long been established. While definite evidence of the parenteral or local application of female sex hormones in the regenerative activity of the hair papillae in humans is still lacking, nevertheless some gonadal or pituitary relationship should be considered. In the present study evidence was brought forward indicating not only that estrogenic hormones produced an increased activity of the hair papillae, but the result of such stimulation was evident in the increased weight of the clipped hair in contrast to the lesser weight of a given clipped area in the control animals and in those animals which were treated with male sex hormones. The fact that the increase of hair occurred not only on the inuncted area, but on the opposite side as well, indicates a general rather than a local effect (fig. 9). It should be pointed out also that the effect of estrogenic hormones is reversible in relationship to the growth of hair, not only on castrated but also on non-castrated animals. There appeared to be no carcinogenic effects of the sex hormones or tumor formation during the conduct of this study.

SUMMARY AND CONCLUSIONS

Percutaneous application of estrogens in rabbits causes a reduction in size of the sebaceous glands, and stimulation of the hair papillae with an increase in rate of growth of the hair.

With percutaneously applied testosterone there was noted hyperplasia of the sebaceous glands and reduced activity of the pilar papillae (less mitotic figures) with a decrease in the rate of growth of hair.

The above features are reversible, i.e., after the application of testosterone for a certain length of time, a reversible effect can be obtained with the use of estrogens, and vice versa.

Obvious changes occur in the cortex of the adrenal glands. These may be observed for as long as 4 months after cessation of the hormones but to a lesser degree than in the first month after continuous application.

Similar changes, although to a lesser degree, also occur after the application of androgenic hormones.

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